โพรไบโอติกสายพันธุ์ *Limosilactobacillus reuteri* TF7 ช่วยป้องกันภาวะไขมันในเลือดผิดปกติและการอักเสบผ่านการปรับสมดุลจุลินทรีย์ในลำไส้ในหนูอ้วนที่ถูกเหนี่ยวนำด้วยอาหารไขมันสูง

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บทคัดย่อ

ภาวะไขมันในเลือดผิดปกติ เป็นภาวะที่ระดับคอเลสเตอรอลและไตรกลีเซอไรด์ในเลือดสูงกว่าปกติ ซึ่งส่งผลเสียต่อสุขภาพในหลายด้าน โดยอาจมีสาเหตุจากภาวะเครียดออกซิเดชันและการอักเสบที่เพิ่มสูงขึ้น ส่งผลให้เกิดโรคต่างๆ เช่น โรคอ้วน โรคเบาหวาน และโรคไขมันพอกตับ มีหลายการศึกษาที่แสดงให้เห็นว่าโพรไบโอติกเป็นจุลินทรีย์มีชีวิตที่มีประโยชน์ต่อสุขภาพ โดยเฉพาะอย่างยิ่งในการลดระดับไขมันในเลือด และช่วยรักษาสมดุลของการเผาผลาญในร่างกาย สำหรับการศึกษานี้ ต้องการศึกษาประสิทธิภาพของโพรไบโอติกสายพันธุ์ *Limosilactobacillus reuteri* TF7 ในการลดระดับไขมัน ลดระดับสารสื่อการอักเสบ การต้านสารอนุมูลอิสระ การปรับภูมิคุ้มกันเฉพาะที่ของลำไส้ และการรักษาสมดุลจุลินทรีย์ในลำไส้ของหนูอ้วนที่ถูกเหนี่ยวนำด้วยอาหารไขมันสูง จากการศึกษาพบว่าโพรไบโอติกสายพันธุ์ *L. reuteri* TF7 สามารถลดน้ำหนักตัว ระดับน้ำตาล และระดับไขมันในกระแสเลือดได้ พร้อมทั้งกระตุ้นการทำงานของเอนไซม์ cholesterol-7α-hydroxylase นอกจากนี้ *L. reuteri* TF7 ยังช่วยลดระดับเอนไซม์ตับและสารสื่อการอักเสบ ได้แก่ TNF-α และ IL-6 ซึ่งส่งผลให้การสะสมไขมันในตับลดลง โพรไบโอติกยังช่วยลดความเครียดออกซิเดชันโดยกระตุ้นการสร้างสารต้านอนุมูลอิสระเพิ่มสูงขึ้น ได้แก่ superoxide dismutase (SOD) และ glutathione peroxidase (GPX) อีกด้วย อีกทั้งการศึกษาครั้งนี้ยังพบว่าโพรไบโอติกสามารถเสริมสร้างภูมิคุ้มกันเฉพาะที่ของลำไส้ได้สองทาง โดยการเพิ่มการแสดงออกของโปรตีน zonula occludens-1 (ZO-1) และลดการแสดงออกของโปรตีน toll-like receptors (TLRs) นอกจากนี้ *L. reuteri* TF7 ยังช่วยปรับสมดุลจุลินทรีย์ในลำไส้โดยเพิ่มกลุ่มประชากรของแบคทีเรียตัวดี การศึกษาในครั้งนี้ชี้ให้เห็นว่าโพรไบโอติกสายพันธุ์ *L. reuteri* TF7 เป็นโพรไบโอติกที่มีศักยภาพในการลดปัจจัยเสี่ยงที่เกี่ยวข้องกับภาวะไขมันในเลือดผิดปกติได้อย่างมีประสิทธิภาพ

คำสำคัญ: ลิโมซิแล็กโทบาซิลลัส รียูเทอรี; โพรไบโอติก; โรคอ้วน; ภาวะไขมันในเลือดผิดปกติ; การอักเสบ

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Probiotic *Limosilactobacillus reuteri* TF7 protects against dyslipidemia and inflammation via modulating gut microbiota in high-fat-diet-induced obese rats

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Abstract

Dyslipidemia is a condition characterized by abnormally high levels of cholesterol and triglycerides. It has numerous negative health effects, which are partly due to increased levels of oxidative stress and inflammation. The resulting health problems include obesity, diabetes, and non-alcoholic fatty liver disease (NAFLD). Several studies have shown that probiotics are beneficial live microorganisms that can promote overall health and well-being, particularly through their lipid-lowering properties and supportive role in maintaining metabolic balance. Thus, this study aimed to investigate the effects of the probiotic *Limosilactobacillus reuteri* TF7 on lipid-lowering; inflammatory mediators; antioxidant activities; intestinal local immunity; and gut microbiota balance in high-fat-diet-induced obese rats. The study showed that oral administration of the probiotic resulted in body weight loss, lower blood glucose and lipid levels, and elevated cholesterol-7α-hydroxylase activity. In addition, *L. reuteri* TF7 reduced liver enzyme levels and pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which led to decreased liver fat accumulation. The probiotic reduced oxidative stress by boosting antioxidant enzyme production, including superoxide dismutase (SOD) and glutathione peroxidase (GPX). The research demonstrated two approaches to enhancing intestinal local immunity: increased expression of the zonula occludens-1 (ZO-1) protein and reduced expression of TLR proteins. The gut microbiota achieved balance through *L. reuteri* TF7, which promoted the growth of beneficial bacteria. Our findings indicate that the probiotic strain *L. reuteri* TF7 has the potential to effectively reduce risk factors associated with dyslipidemia.

Keywords: *Limosilactobacillus reuteri*; probiotics; obesity; dyslipidemia; inflammation

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Introduction

Dyslipidemia is a condition in which blood lipid levels become abnormal, leading to elevated cholesterol and triglyceride concentrations. These abnormalities can cause obesity and diabetes and contribute to cardiovascular disease and non-alcoholic fatty liver disease (NAFLD)[1-2]. Dyslipidemia also leads to the accumulation of visceral fat, resulting in elevated levels of free fatty acids (FFAs). These FFAs can induce the release of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)[3]. Moreover, increased levels of unsaturated fatty acids can react with reactive oxygen species (ROS) to cause lipid peroxidation that results in malondialdehyde (MDA) secretion as an oxidative end product[4]. Elevated MDA levels create oxidative stress, which can lead to various health issues. It has been reported that dyslipidemia is associated with alterations in the structure and function of the gut microbiota[5]. Dyslipidemia can disrupt the balance of the gut microbiota, allowing harmful bacteria to proliferate and triggering inflammatory and immune responses through the activation of toll-like receptors (TLRs). This process weakens intestinal barrier integrity, leading to a "leaky gut." This condition may allow lipopolysaccharides (LPS) to enter the bloodstream, causing chronic inflammation and lipid metabolism disorders[6-7]. Although statins and other lipid-lowering drugs effectively treat dyslipidemia, their long-term use can cause side effects such as muscle pain, hepatotoxicity, and diabetes[8].

Probiotics consist of live microorganisms that help restore and maintain gut microbiota health, thereby improving total body wellness. Several studies have demonstrated that probiotics are safe and have no adverse effects while promoting health benefits[9]. They serve as a supportive approach for preventing and improving diet-induced lipid metabolism disorders and related chronic diseases[10]. Probiotics help lower lipid levels by producing bile salt hydrolase (BSH), an enzyme that converts bile salts into deconjugated forms that are less soluble in water. These deconjugated bile salts are excreted through feces, prompting the body to use cholesterol to synthesize new bile salts, which results in decreased blood lipid levels[11]. *Lactobacilli* are a major component of the human gut microbiota and represent one of the most commonly used and studied probiotics[12]. Many *Lactobacillus* strains have been shown to exert cholesterol-lowering effects in the host and to help reduce inflammation and liver damage associated with obesity[13-14]. *Limosilactobacillus* is one of the new genera that were separated from the original *Lactobacillus* in 2020[15]. In addition, various *Limosilactobacillus* strains contain genes encoding BSH, and numerous studies have demonstrated their beneficial effects on dyslipidemia-related conditions in animal models[16-17]. For example, *L. fermentum* HNU312 improved lipid metabolism and gut microbiota balance, reducing body weight, lipid levels, and inflammation caused by a high-fat diet[18]. Furthermore, *L. reuteri* suppressed the

proinflammatory cytokine TNF-α while enhancing the anti-inflammatory cytokine interleukin-10 (IL-10), with these effects associated with the TLR4 response[19]. Finally, gut microbiota imbalance was improved by supplementation with a *L. reuteri* strain[20].

Limosilactobacillus reuteri TF7, isolated from traditional Thai food, may be well suited for protecting against dyslipidemia and related conditions, as it exhibits strong BSH activity and effectively reduces lipid levels in rats[21-22]. This study aimed to build upon previous findings on this strain's anti-dyslipidemic potential by thoroughly evaluating its effects on lipid regulation, inflammation, antioxidant activity, intestinal local immunity, and gut microbiota balance in high-fat-diet-induced obese rats.

Materials and Methods

Ethical approval

All animal experiments were conducted in accordance with institutional and national ethical guidelines to ensure animal welfare through proper care practices. The study protocol was reviewed and approved by the Animal Ethics Committee of Srinakharinwirot University (approval number: COA/AE-004-2564).

Preparation of probiotic strain and high-fat diet

The *L. reuteri* TF7[21] strain, a probiotic isolated from traditional Thai foods, was supplied by the Center of Excellence in Probiotics, Faculty of Medicine, Srinakharinwirot University, Thailand. *L. reuteri* TF7 was prepared for the animal study using standard microbiological methods. The bacteria were thawed from -80 °C storage before being grown on de Man, Rogosa, and Sharpe (MRS) agar (HiMedia, India) at 37 °C under anaerobic conditions for 48 hours. The colonies were subsequently subcultured three times to achieve both purity and stable culture conditions. The cells were harvested by centrifugation at 4,000 × *g* for 5 minutes at 4 °C to separate them from the MRS broth, then washed and resuspended in PBS solution at a concentration of 10^9 CFU/mL.

The high-fat diet (HFD), a modified formulation derived from Puttarat et al.[22], contained the following components (% w/v): 40% fresh egg yolk, 17.2% beef tallow, 16.25% egg yolk powder, 12.5% sucrose, 5% cholesterol powder, and 0.5% sodium cholate. The composition of this diet provided 487.05 kcal of metabolizable energy per 100 g of food. The basal diet (control), obtained from the National Laboratory Animal Center at Mahidol University Thailand, contained 52% carbohydrates, 24% protein, 5% fiber, and 4.5% fat, providing 146.5 kcal of total metabolizable energy per 100 g.

Animal experiment and feeding protocol

Male Sprague-Dawley rats aged eight weeks and weighing 250-280 g were purchased from Nomura Siam International Co., Ltd. (Thailand). The Medical Center Animal Care Laboratory at Srinakharinwirot University maintained the animals under laboratory conditions with environmental settings of 22 ± 2 °C, 55 ± 10% relative humidity, and a 12-h light/dark cycle. After a one-week acclimatization period, the rats were randomly assigned to three experimental groups (n = 5

per group based on a previous study[22]): (1) normal control (NC), (2) high-fat diet control (HFD), and (3) HFD with *L. reuteri* TF7 supplementation (HLR7).

The animal experiment was conducted over 12 weeks. During weeks 1–4, the NC group received 2 mL of phosphate-buffered saline (PBS) daily by oral gavage. The HFD group was administered 1 mL of PBS and 1 mL of the HFD mixture, whereas the HLR7 group received 1 mL of *L. reuteri* TF7 suspension (10^9 CFU/mL in PBS) together with 1 mL of HFD. From weeks 5-12, all treatments were provided at twice the initial dosage. The experimental design and feeding regimen are illustrated in Figure 1A.

Throughout the experimental period, all animals had free access to the basal diet and water. Body weight, food intake, and water consumption were recorded weekly, and fecal samples were collected at the end of the study. Following a 12-h fasting period, all rats were anesthetized with isoflurane (Piramal Critical Care, USA) and euthanized. Blood samples were collected by cardiac puncture into NaF-treated and heparinized tubes. The liver, abdominal fat, and colon tissues were collected for subsequent analyses. The liver and abdominal fat were weighed immediately after collection. Finally, the liver index and abdominal fat index were calculated as the ratio of the wet organ weight to the final body weight.

Biochemical analysis

Blood samples were promptly centrifuged at 4,000 × *g* for 10 min at 4 °C to obtain plasma. The separated plasma samples were analyzed for total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). All analyses were performed by Professional Laboratory, Thailand (PROLAB).

Histological analysis

Liver tissue samples were fixed in 4% paraformaldehyde solution at 4 °C overnight, followed by ethanol dehydration for 1-2 hours. The tissue samples were embedded in paraffin, and 3-5 µm-thick sections were cut using a microtome (HistoCore Multicut, Leica Biosystems, Germany). The tissue sections were stained with hematoxylin and eosin (H&E) and Oil Red O, followed by microscopic examination under a light microscope (Olympus UC50, Japan) in 10 randomly selected fields at 400× magnification. The NAS (Nonalcoholic Fatty Liver Disease Activity Score)[23] tool was used to evaluate liver injury associated with obesity. The NAS score ranges from 0 to 8 and represents the combined score of steatosis (0-3), lobular inflammation (0-3), and hepatocyte ballooning (0-2).

For semi-quantitative evaluation, colon tissues were processed as described above and stained with H&E. Ten randomly chosen areas were examined under an Olympus UC50 light microscope at 200× magnification. The Geboes Score (GS)[24] was used to evaluate histopathological damage by

assessing erosions or ulcerations, mononuclear cell infiltration, tissue architecture, and crypt integrity. The scoring system ranges from 0 to 5, representing no abnormality at 0, immune cells in the lamina propria at 1-2, epithelial infiltration at 3, crypt destruction at 4, and the most severe condition of severe erosion or ulceration at 5. Paracellular permeability in the ascending colon was assessed using immunohistochemical methods to detect the tight junction protein zona occludens-1 (ZO-1) by applying polyclonal antibodies (61-7,300; Invitrogen).

Assessment of hepatic cholesterol-7α-hydroxylase (CYP7A1) level

Liver tissues were homogenized (10% w/v) in RIPA lysis buffer (Sigma-Aldrich, USA). The homogenates were then sonicated on ice using an ultrasonic homogenizer (Sonoplus, Germany) for 3-5 min at a power output of 25-30% (approximately 180 W). The resulting lysates were centrifuged at 300 x *g* for 10 min at 0 °C, and the supernatants were collected for subsequent analyses.

CYP7A1 levels in the homogenized liver tissues were quantified using a CYP7A1 enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's protocol (MyBioSource, San Diego, CA, USA).

Assessment of anti-inflammation activities in liver tissues

Homogenized liver samples were used to quantify levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) using enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen, Waltham, MA, USA) according to the manufacturer's protocol.

Assessment of antioxidant activities in liver tissues

The levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX) in homogenized liver tissues were determined using commercially available assay kits (Cayman Chemical, Ann Arbor, MI, USA) following the manufacturer's instructions.

Quantitative real-time PCR

Quantitative real-time PCR was conducted to analyze the expression of genes that regulate inflammatory responses and bile acid production. Total RNA was isolated from liver and colon tissues using TRIzol reagent (Thermo Fisher Scientific, USA). Complementary DNA (cDNA) was synthesized from the extracted RNA using a reverse transcriptase kit (Applied Biosystems, UK). Quantitative PCR was performed on the QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, USA) with SYBR® Green PCR Master Mix. The β-actin gene was used as an internal control to normalize the relative expression levels of target genes. Primer sequences are presented in Table 1.

Table 1 Primer sequences used in this study

Gene	Forward	Reverse
Cytochrome P450 7A1 (CYP7A1)	5'-TGCCGGTACTAGACAGCATC-3'	5'-TCCTCCTTAGCTGTGCGGAT-3'
Toll-like receptor 2 (TLR-2)	5'-CGCTTCCTGAACTTGTCC-3'	5'-GGTTGTCACCTGCTTCCA-3'
Toll-like receptor 4 (TLR-4)	5'-GCATCATCTTCATTGTCCTTGAGA-3'	5'-CTCCCACTCGAGGTAGGTGTTT-3'
β*-actin*	5'-ACTGCCCTGGCTCCTAGCA-3'	5'-GCCAGGATAGAGCCACCAATC-3'

Intestinal microbiota analysis

Fecal samples were collected at the end of the experiment for microbiome profiling using next-generation sequencing (NGS). DNA was extracted with the QIAamp Fast DNA Stool Mini Kit (Qiagen, USA) following the manufacturer's protocol. DNA concentration and purity were measured by using a NanoDrop 2,000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V3-V4 variable regions of the bacterial 16S rRNA gene were amplified and sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).

Bioinformatic analysis was performed using Quantitative Insights Into Microbial Ecology version 2.0 (QIIME2) software, where high-quality sequences were clustered into Operational Taxonomic Units (OTUs) at 97% similarity against the Silva database. The Shannon index was used to assess alpha-diversity, while beta-diversity was evaluated using UniFrac distances and visualized via Principal Coordinate Analysis (PCoA).

Statistical analysis

All experiments were conducted in triplicate, and the results were presented as means ± standard deviation (SD). Statistical analysis was performed using GraphPad version 10.1.0 (San Diego, USA). Data normality was assessed using the Shapiro–Wilk test. Differences between groups were analyzed using one-way ANOVA followed by Tukey's multiple comparison test; if the data in at least one group were not normally distributed, the Kruskal–Wallis test with Dunn's post-test was used, as shown in the figure legends. Statistical significance was defined as a p-value < 0.05.

Results

Body weight, body weight gain, and visceral tissue indices

To evaluate the effects of probiotics on physiological abnormalities associated with dyslipidemia, body weight, body weight gain, food and water intake, and visceral tissue indices were assessed in rats fed a high-fat diet (HFD). As shown in Figure 1, body weight increased in a time-dependent manner in all three experimental groups over the 12-week study period. Rats in the HFD control group showed the greatest and most significant weight gain compared with the normal control (NC) group. Conversely, supplementation with *L. reuteri* TF7 (HLR7) significantly reduced body weight in HFD-fed rats compared with the HFD

group (Figure 1B, C). Moreover, no significant differences in food or water intake were observed among the experimental groups (data not shown). The results for abdominal fat weight and fat index were consistent with the body weight findings, showing significantly higher values in the HFD group than in the NC and HLR7 groups (Figure 1D, E). In contrast, no significant differences were observed among the groups with respect to liver indices (Figure 1F, G).

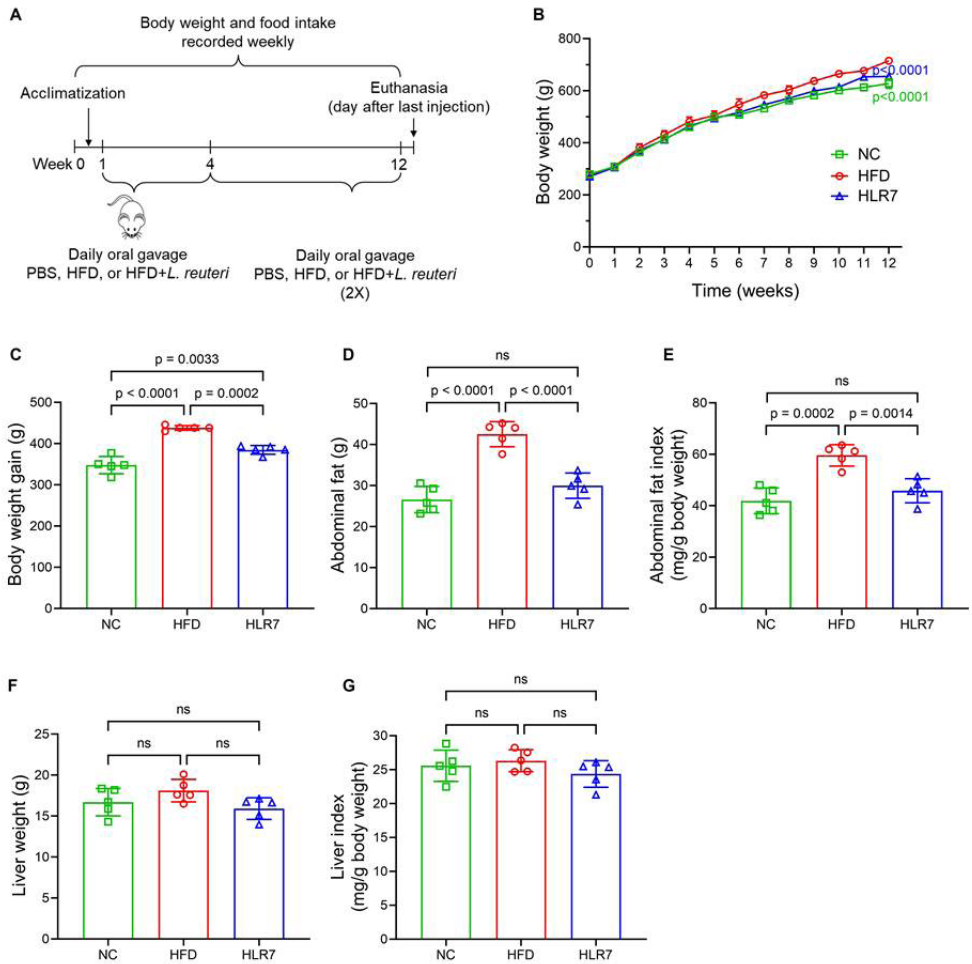

Figure 1 Experimental design of the obese rat model and the effects of *L. reuteri* TF7 supplementation on body weight and visceral tissue indices. (A) Experimental protocol: rats fed a daily oral gavage of PBS (NC), a high-fat diet (HFD), or an HFD supplemented with *L. reuteri* TF7 (HLR7) at a concentration of 10^9 CFU/mL. From weeks 5-12, the dosage was doubled. (B) Weekly body weight, (C) Body weight gain, (D) Abdominal fat weight, (E) Abdominal fat index, (F) Liver weight, and (G) Liver index. (A-G) n = 5 per group. One-way ANOVA followed by Tukey's post-test was used. Error bars represent mean ± SD.

Lipid profile, blood glucose levels, and liver histology

To determine the effect of *L. reuteri* TF7 supplementation on lipid metabolic disorders associated with dyslipidemia, important parameters related to dyslipidemia were measured, including lipid profile (TC, TG, LDL-C, and HDL-C) and FBG. Additionally, liver tissue was examined for *CYP7A1* gene expression and protein levels, which represent the main regulatory enzyme of bile acid biosynthesis within the cytochrome P450 family responsible for removing cholesterol from the body. The levels of TC, TG, and LDL-C were significantly increased compared with the normal control, whereas HDL-C levels were decreased in rats fed the HFD. *L. reuteri* TF7 was able to restore TC, TG, and LDL-C levels almost to control levels; however, no significant improvement was observed in HDL-C levels (Figures 2A-D). The HFD group exhibited a significant elevation in FBG levels, which were fully restored to normal control values following *L. reuteri* TF7 supplementation (Figure 2E). Finally, both CYP7A1 protein and gene expression were significantly upregulated in the HLR7 group compared with the HFD group (Figure 2F, G).

High blood lipid levels activate lipolysis, leading to liver fat accumulation and resulting in hepatic steatosis. Therefore, liver histology was examined. The hepatocytes in liver tissue were stained with H&E. The morphology of cells from the NC and HLR7 groups appeared normal. The cells were spherical with defined boundaries, and the nuclei were situated at their centers. In contrast, hepatocytes from the HFD group showed abnormal ballooning morphology with enlarged nuclei and increased intracellular lipid droplets (Figure 3A). Moreover, the Oil Red O staining results showed that the HFD group accumulated more lipids than the NC and HLR7 groups (Figure 3A), and the HFD group had a significantly higher liver injury score than the other groups (Figure 3B).

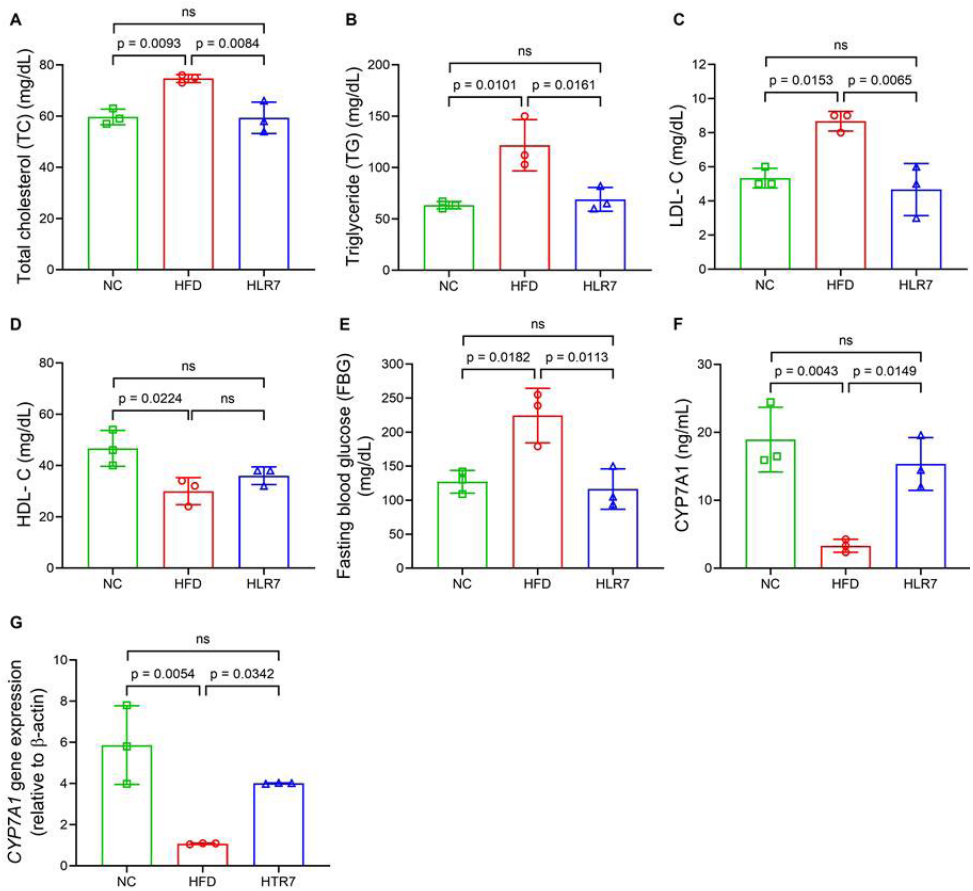

Figure 2 The effects of *L. reuteri* TF7 supplementation on lipid profile, fasting blood glucose, and hepatic cholesterol-7α-hydroxylase levels in rats at the end of the experiment. (A) Total cholesterol (TC), (B) Triglyceride (TG), (C) LDL-C, (D) HDL-C, (E) Fasting blood glucose (FBG), (F) CYP7A1 protein level, and (G) *CYP7A1* gene expression of rats in each group. (A-G) n = 3 per group. One-way ANOVA testing followed by Tukey's post-test analysis was used. Error bars represent mean ± SD.

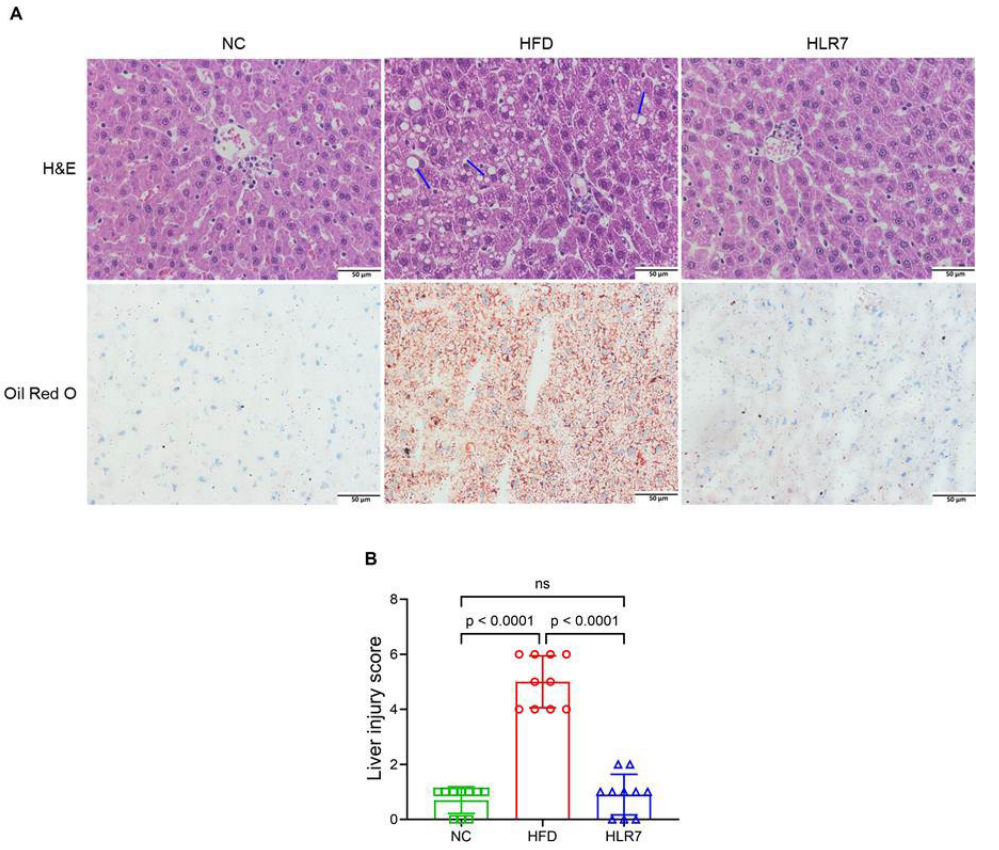

Figure 3 The effects of *L. reuteri* TF7 supplementation on liver histology. (A) Photomicrographs of rat liver sections stained with H&E and Oil Red O; blue arrows indicate fat droplets. (B) Liver injury scores. n = 10 per group. One-way ANOVA followed by the Kruskal-Wallis test with Dunn's post-test was used. Error bars represent mean ± SD.

Anti-inflammatory and antioxidants activities

Organ damage from dyslipidemia occurs due to elevated blood lipid levels. The liver is one of the primary organs affected, and AST and ALT enzymes serve as key indicators of liver damage and inflammation. The HFD group exhibited significantly higher AST and ALT levels than the NC group, whereas supplementation with *L. reuteri* TF7 significantly reduced these enzymes to near-control values (Figure 4A, B). The cytokine results also demonstrated the anti-inflammatory effects of *L. reuteri* TF7. HFD feeding significantly elevated pro-inflammatory cytokines (TNF-α and IL-6) while suppressing anti-inflammatory IL-10 levels compared to the NC group. Following *L. reuteri* TF7 supplementation, these cytokine levels were restored toward normal (Figure 4C-E).

Consistently, *L. reuteri* TF7 supplementation also enhanced antioxidant defenses, as indicated by reduced malondialdehyde (MDA) levels, a key biomarker of oxidative stress, and increased activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) antioxidant enzymes in liver tissue (Figure 4F-H).

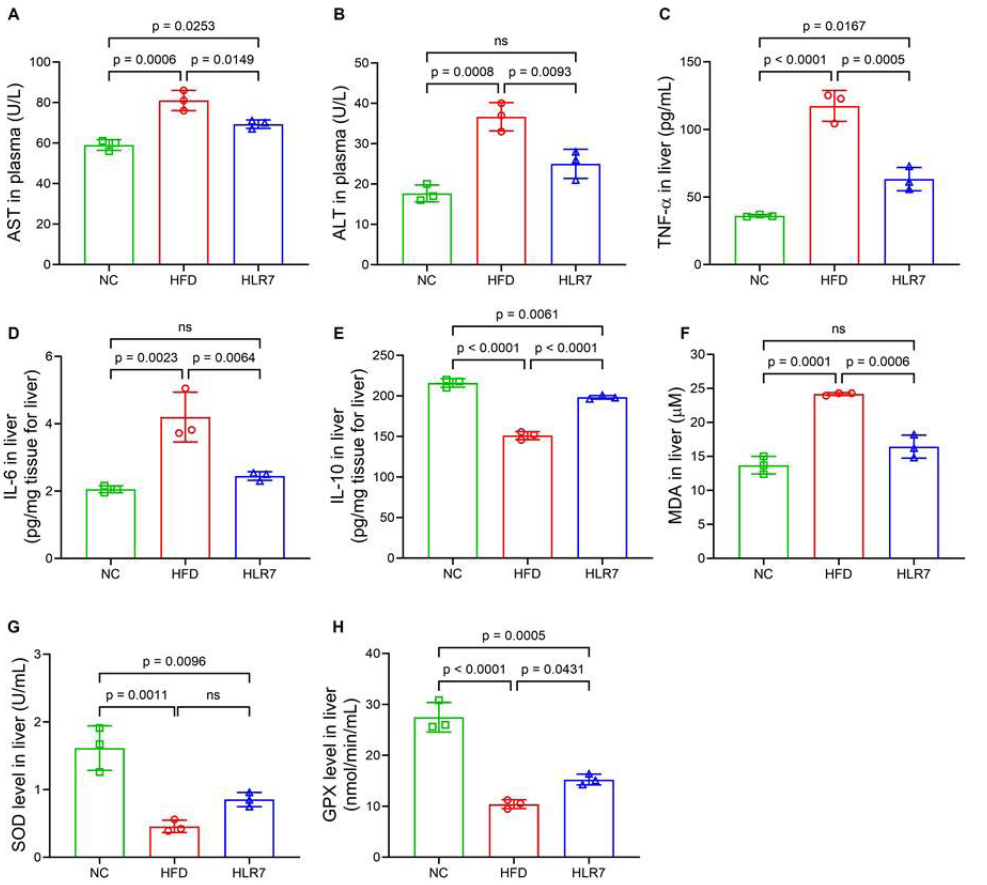

Figure 4 The effects of *L. reuteri* TF7 supplementation on anti-inflammatory and antioxidant activities. (A) AST (aspartate transaminase) and (B) ALT (alanine transaminase) in plasma. (C) TNF-α, (D) IL-6, (E) IL-10, (F) MDA, (G) SOD, and (H) GPX in liver tissue. (A-H) n = 3 per group. One-way ANOVA followed by Tukey's post-test was used. Error bars represent mean ± SD.

Intestinal inflammation and local immunity

The intestinal epithelium functions as the main physical and immune barrier. Its integrity depends on zonula occludens (ZO) tight junction proteins, which connect cells while controlling the passage of substances between them. Toll-like receptors (TLRs) play a key role in local immune responses against pathogens, and their expression is increased during inflammation. Dyslipidemia can impair the intestinal barrier, resulting in structural damage and functional deficits.

To evaluate structural damage to the intestinal barrier, colon tissue morphology was examined using H&E staining, and the tight junction protein zonula occludens-1 (ZO-1) was detected using immunohistochemistry. The HFD group showed intestinal epithelial damage characterized by severely disrupted crypts and mononuclear cell infiltration in both the lamina propria and epithelial layers. In contrast, the NC and HLR7 groups displayed normal epithelial architecture without mononuclear cell infiltration, as shown in Figure 5A. These findings corresponded with the colon injury scores, which were strongly elevated only in the HFD group (Figure 5B). Moreover, ZO-1 expression was higher in the HLR7 group than in the HFD group, reaching levels comparable with the control (Figure 5C). Furthermore, a high-fat diet affects the diversity of intestinal microbiota and local immune responses in the intestinal barrier. In this study, the expression of TLR-2 and TLR-4 was measured; these receptors are part of the local immune response to gram-positive and gram-negative bacteria, respectively, during inflammation. A significant upregulation of TLR-4 expression was observed in the HFD group, whereas TLR-2 levels remained unchanged relative to the NC group. The *L. reuteri* TF7-supplemented group showed a significant decrease in TLR-2 and TLR-4 expression compared with the HFD group (Figure 5D, E).

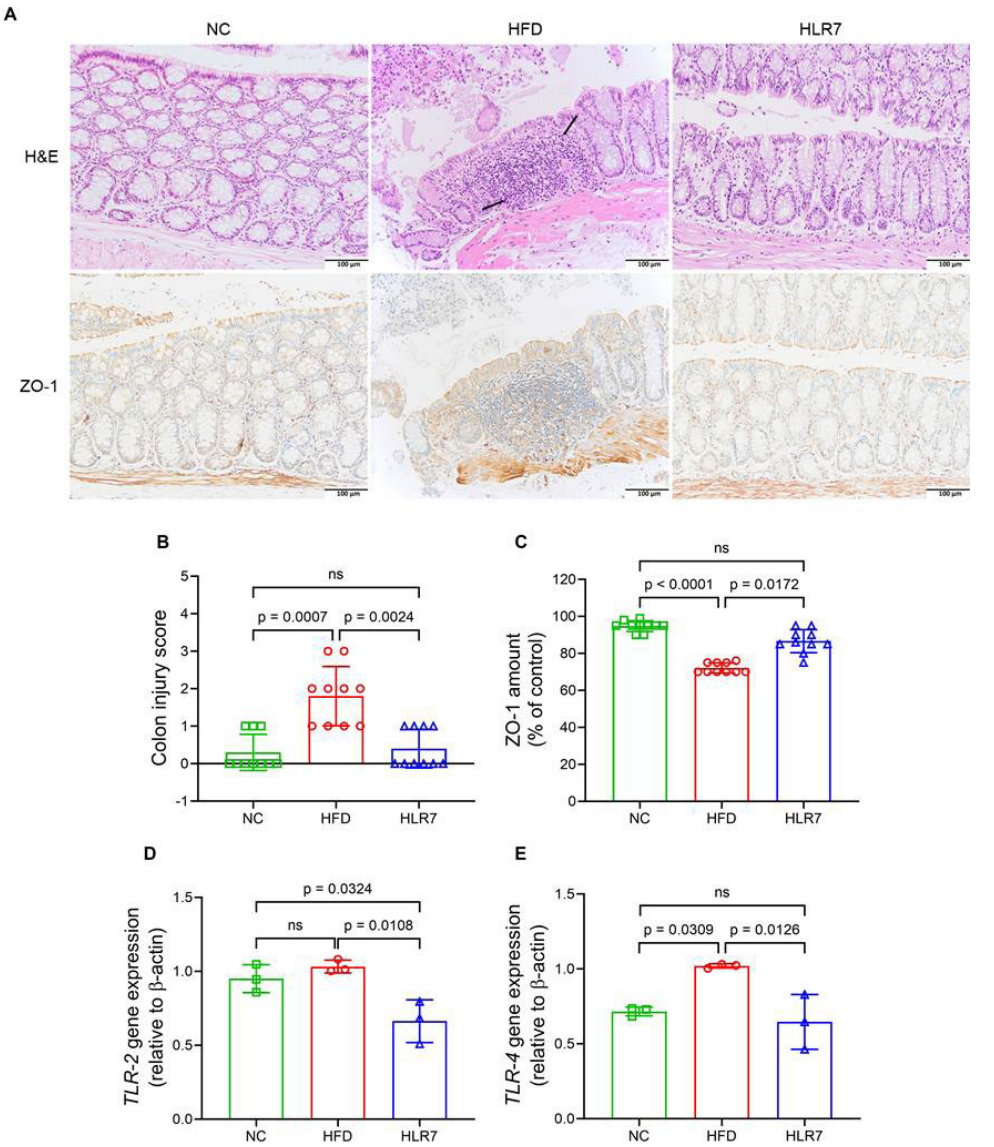

Figure 5 The effects of *L. reuteri* TF7 supplementation on intestinal inflammation and local immunity. (A) Representative H&E-stained images of colon tissues and immunohistochemical staining for ZO-1. Black arrows indicate immune cell infiltration. (B) Colon injury score. (C) Relative ZO-1 levels (% of control). (D) *TLR-2* and (E) *TLR-4* gene expression relative to β-actin. (B, C) n = 10 per group; statistical significance was determined using one-way ANOVA followed by the Kruskal–Wallis and Dunn's post-tests. (D, E) n = 3 per group; analysis was performed using Tukey's post-test. Error bars represent mean ± SD.

Intestinal microbiota

To evaluate the impact of the probiotic *L. reuteri* TF7 on intestinal microbiota, fecal samples from each rat group were analyzed. The HFD group showed a significant decrease in the Shannon index (α diversity) compared with the NC group. However, rats receiving *L. reuteri* TF7 also showed a significant decrease in α diversity (Figure 6A). β-diversity principal component analysis revealed a distinct microbial composition in the HFD group, as shown in the PCoA plot, which differed from the NC and HLR7 groups (Figure 6B). At the phylum, genus, and species levels, notable differences were found between the HFD and NC groups. The HFD group showed an increased relative abundance of *Firmicutes*, *Lachnospiraceae*, and *Peptococcaceae*, whereas these changes were reversed in the HLR7 group (Figure 6C-E).

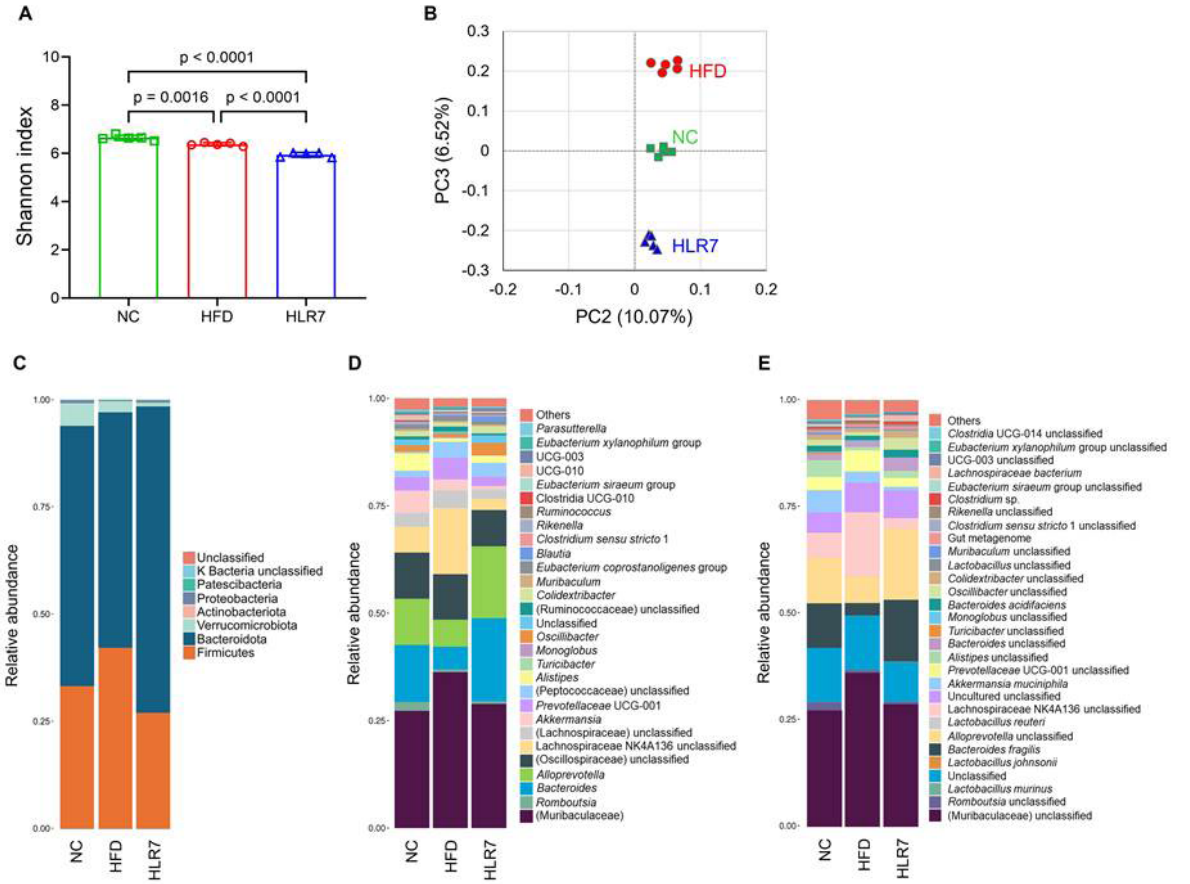

Figure 6 The effects of *L. reuteri* TF7 supplementation on the intestinal microbiome. (A) Shannon index (α-diversity); statistical analysis was performed using one-way ANOVA followed by Tukey's post-test. Error bars represent mean ± SD. (B) Principal component analysis (PCoA; β-diversity). Relative abundances of OTUs at the (C) phylum, (D) genus, and (E) species levels. (A, B) n = 5 per group.

Discussion

Probiotics have become widely accepted because they provide multiple health benefits to humans. They have shown potential in helping to manage immune system disorders, inflammation, oxidative stress, and gut microbiota imbalances. Moreover, previous studies show that probiotic mixtures[25] and individual strains[26] from *Lactobacillus* and *Bifidobacterium* genera help regulate abnormal lipid metabolism, which contributes to dyslipidemia and non-communicable diseases (NCDs). Previous studies showed that the probiotic strain *Limosilactobacillus reuteri* TF7 lowers lipid levels due to its BSH enzyme activity[21]. It has also been reported to reduce body weight and modulate the gastrointestinal microbiota[22], suggesting that it may help alleviate conditions induced by an HFD. Building on these findings, our study aimed to further confirm and better understand these effects.

In this study, HFD group rats demonstrated substantial weight gain, and their lipid profiles and FBG levels were significantly elevated. The main substance of high-fat diets, which is fat, leads to dyslipidemia, abdominal fat accumulation, and other pathological effects. Elevated free fatty acids (FFAs) in dyslipidemia cause insulin receptor substrate-1 blockage, resulting in insulin resistance, reduced glucose uptake, and elevated blood glucose levels[27]. After 12 weeks of supplementation with *L. reuteri* TF7, body weight gain and abdominal fat index were significantly reduced. As anticipated, *L. reuteri* TF7 decreased blood glucose levels, and the lipid-lowering effect was indicated by significant reductions in total cholesterol, triglyceride, and LDL-C levels. HDL-C was measured but showed no significant change following *L. reuteri* TF7 treatment. This may be due to the characteristics of specific strains, as a previous study reported that *L. reuteri* TF7 did not significantly improve HDL-C levels in hypercholesterolemic rats[22]. Notably, the expression levels of the CYP7A1 enzyme and gene, which are important rate-limiting steps in bile acid synthesis from cholesterol[28], were significantly increased. This suggests that the reduction in lipid levels may be linked to enhanced bile acid metabolism mediated by BSH activity, as shown in previous studies. These findings are consistent with the liver histological analysis, as the liver is a key organ associated with the detrimental effects of dyslipidemia. In dyslipidemia-induced rats, lipid droplets accumulate in hepatocyte cytoplasm, causing liver cells to balloon and enlarge, which can progress to non-alcoholic fatty liver disease (NAFLD) due to a high-fat diet[29]. In contrast, normal hepatocytes were observed in the normal control and *L. reuteri* TF7 groups. Thus, *L. reuteri* TF7, as a probiotic, may help reduce fat infiltration in the liver and improve liver damage caused by dyslipidemia.

Excessive cholesterol accumulation in liver cells contributes to oxidative stress, which increases liver injury and inflammation. AST and ALT are key markers of liver damage, as they are released into the bloodstream when hepatocytes are damaged[30]. Our results showed that, after treatment with *L. reuteri*

TF7, AST and ALT levels were significantly reduced. Similarly, treatment with *L. fermentum* CQPC07 greatly lowered the increases in AST and ALT levels caused by an HFD[14]. When the liver becomes injured and inflamed, immune cells release pro-inflammatory cytokines such as TNF-α and IL-6[31]. In this study, we observed a significant decrease in TNF-α and IL-6 levels and an increase in the anti-inflammatory cytokine IL-10, which helps regulate the inflammatory process, following supplementation with *L. reuteri* TF7. Lipid peroxidation occurs when free radicals react with polyunsaturated fatty acids from accumulated fat, leading to the production of reactive compounds like MDA, a marker of oxidative stress[32]. To protect against this damage, the body activates antioxidants such as SOD and GPX. Increased levels of MDA, along with reduced levels of SOD and GPX, were observed in HFD-induced rats. After supplementation with *L. reuteri* TF7, these levels were restored, as several probiotics inhibit the production of ROS and promote antioxidative enzymes through the upregulation of GPX, SOD, and glutathione (GSH)[33]. From these findings, the probiotic *L. reuteri* TF7 was effective in improving liver injury and inflammation caused by dyslipidemia, reducing lipid accumulation, and enhancing liver health by lowering pro-inflammatory cytokines, increasing anti-inflammatory cytokines, and reducing oxidative stress through inhibition of lipid peroxidation and increased antioxidant enzyme activity.

Dyslipidemia disrupts intestinal structure and endothelial barrier function because intestinal epithelial cells play a crucial role in maintaining physical and immune defense functions. In this study, the expression of ZO-1, a tight junction protein that helps maintain the integrity of intestinal epithelial cells and regulates paracellular permeability, was measured and found to be reduced in the HFD group. This result corresponded with colon histology, which showed immune cell infiltration in the epithelial layers and a loss of crypts and goblet cells. Conversely, rats supplemented with *L. reuteri* TF7 exhibited normal colon morphology, similar to the control group, along with higher ZO-1 expression. Probiotics help reduce inflammation by protecting intestinal epithelial cells from damage and enhancing the expression of tight junction proteins[34]. Additionally, probiotics play an important role in regulating the expression of TLRs, which are key immune receptors in intestinal epithelial cells. Pathogenic bacteria more actively trigger the TLR-2 and TLR-4 receptors[35]. However, the activation of TLRs is closely linked to the gut microbiota. This study indicated that oral administration of *L. reuteri* TF7 significantly reduced the expression of TLR-2 and TLR-4, similar to findings in HFD-fed rats treated with *L. plantarum* S9, which also showed downregulation of TLR-4 expression and a reduction in inflammatory factor levels[36].

Previous studies have shown that dyslipidemia causes major changes in both gut microbiota diversity and function[37]. In this study, no significant difference was observed in the ratio of *Firmicutes* to *Bacteroidetes*

between the HFD and *L. reuteri* TF7-supplemented groups. Research findings indicate that the *Firmicutes* to *Bacteroidetes* ratio does not serve as a determinant of obesity, according to previous studies[38]. The relative abundance of *Lachnospiraceae* and *Peptococcaceae*, which are associated with dyslipidemia, intestinal inflammation, and obesity, was significantly increased in the HFD group, consistent with previous studies[39-40]. Supplementation with the probiotic strain *L. reuteri* TF7 resulted in decreased levels of *Lachnospiraceae* and *Peptococcaceae*. These findings indicate that the *L. reuteri* TF7 probiotic strain helps restore and improve gut microbiota health associated with dyslipidemia.

Conclusions

In this study, *Limosilactobacillus reuteri* TF7 showed broad beneficial effects on dyslipidemia-related symptoms by improving lipid profiles and FBG levels, reducing hepatic fat accumulation, decreasing liver and intestinal inflammation through enhanced tight junction expression, modulating intestinal immunity, and balancing gut microbiota. These findings suggest that *L. reuteri* TF7 is a potential probiotic strain that can help protect against and reduce risk factors associated with dyslipidemia-related diseases.

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References

1. Mancini GBJ, Hegele RA, Leiter LA. Dyslipidemia. Can J Diabetes 2018;42: S178-85. doi:10.1016/j.jcjd.2017.10.019.
2. Liao X, Ma Q, Wu T, et al. Lipid-lowering responses to dyslipidemia determine the efficacy on liver enzymes in metabolic dysfunction-associated fatty liver disease with hepatic injuries: A prospective cohort study. Diabetes Metab Syndr Obes 2022;15(null):1173-84. doi:10.2147/DMSO.S356371.
3. Zhou H, Urso CJ, Jadeja V. Saturated fatty acids in obesity-associated inflammation. J Inflamm Res 2020;13(null):1-14. doi:10.2147/JIR.S229691.
4. Busch CJ, Binder CJ. Malondialdehyde epitopes as mediators of sterile inflammation. BBA - Molecular and Cell Biology of Lipids 2017;1862(4):398-406. doi:10.1016/j.bbalip.2016.06.016.
5. Jia X, Xu W, Zhang L, et al. Impact of gut microbiota and microbiota-related metabolites on hyperlipidemia. Front Cell Infect Microbiol 2021;11:634780. doi:10.3389/fcimb.2021.634780.
6. Flaig B, Garza R, Singh B, et al. Treatment of dyslipidemia through targeted therapy of gut microbiota. Nutrients 2023;15(1):228. doi:10.3390/nu15010228.

7. Fuke N, Nagata N, Suganuma H, et al. Regulation of gut microbiota and metabolic endotoxemia with dietary factors. Nutrients 2019;11(10). doi:10.3390/nu11102277.
8. Ramkumar S, Raghunath A, Raghunath S. Statin therapy: Review of safety and potential side effects. Acta Cardiol Sin 2016;32(6):631-9. doi:10.6515/acs20160611a.
9. Maftei N-M, Raileanu CR, Balta AA, et al. The potential impact of probiotics on human health: An update on their health-promoting properties. Microorganisms 2024;12(2):234. doi:10.3390/microorganisms12020234.
10. Wang Y, Ai Z, Xing X, et al. The ameliorative effect of probiotics on diet-induced lipid metabolism disorders: A review. Crit Rev Food Sci Nutr 2024;64(11):3556-72. doi:10.1080/10408398.2022.2132377.
11. Song Z, Cai Y, Lao X, et al. Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes based on worldwide human gut microbiome. Microbiome 2019;7(1):9. doi:10.1186/s40168-019-0628-3.
12. Dronkers TMG, Ouwehand AC, Rijkers GT. Global analysis of clinical trials with probiotics. Heliyon 2020;6(7). doi:10.1016/j.heliyon.2020.e04467.
13. Khare A, Gaur S. Cholesterol-lowering effects of *Lactobacillus* species. Curr Microbiol 2020;77(4):638-44. doi:10.1007/s00284-020-01903-w.
14. Wu Y, Li X, Tan F, et al. *Lactobacillus fermentum* CQPC07 attenuates obesity, inflammation and dyslipidemia by modulating the antioxidant capacity and lipid metabolism in high-fat diet induced obese mice. J Inflamm 2021;18(1):5. doi:10.1186/s12950-021-00272-w.
15. Zheng J, Wittouck S, Salvetti E, et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus Beijerinck* 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int J Syst Evol Microbiol 2020;70(4):2782-858. doi:10.1099/ijsem.0.004107.
16. Zhou M, Xiao M, Guo H, et al. The alleviating effect of different bile salt hydrolase gene-encoding *Limosilactobacillus fermentum* strains on hypercholesterolemia in mice. Food Biosci 2025;68:106574. doi:10.1016/j.fbio.2025.106574.
17. Szczepankowka A, Cukrowska B, Aleksandrzak-Piekarczyk T. Complete genome sequence of *Limosilactobacillus reuteri* LU150, a potential vitamin B12 producer from the NORDBIOTIC collection. Microbiol Resour Announc 2024;13. doi:10.1128/mra.00800-24.
18. Li J, Zhang Z, Xu Y, et al. *Limosilactobacillus fermentum* HNU312 alleviates lipid accumulation and inflammation induced by a high-fat diet: Improves lipid metabolism pathways and increases short-chain fatty acids in the gut microbiome. Food Funct 2024;15(17):8878-92. doi:10.1039/d4fo02390k.

19. Luo Z, Chen A, Xie A, et al. *Limosilactobacillus reuteri* in immunomodulation: Molecular mechanisms and potential applications. Front Immunol 2023;14. doi:10.3389/fimmu.2023.1228754.
20. Tang M, Li X, Ren J, et al. *Limosilactobacillus reuteri* HM108 alleviates obesity in rats fed a high-fat diet by modulating the gut microbiota, metabolites, and inhibiting the JAK-STAT signalling pathway. Front Nutr 2025;12. doi:10.3389/fnut.2025.1597334.
21. Puttarat N, Ladda B, Kasorn A, et al. Cholesterol-lowering activity and functional characterization of lactic acid bacteria isolated from traditional Thai foods for their potential used as probiotics. Songklanakarin J Sci Technol 2021;43(5):1283-91. doi:10.14456/sjst-psu.2021.167.
22. Puttarat N, Kasorn A, Vitheejongjaroen P, et al. Beneficial effects of indigenous probiotics in high-cholesterol diet-induced hypercholesterolemic rats. Nutrients 2023;15(12). doi:10.3390/nu15122710.
23. Brunt EM, Kleiner DE, Wilson LA, et al. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: Distinct clinicopathologic meanings. Hepatology 2011;53(3):810-20. doi:10.1002/hep.24127.
24. Geboes K, Riddell R, Ost A, et al. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut 2000;47(3):404-9. doi:10.1136/gut.47.3.404.
25. Mazziotta C, Tognon M, Martini F, et al. Probiotics mechanism of action on immune cells and beneficial effects on human health. Cells 2023;12(1). doi:10.3390/cells12010184.
26. Qiao Y, Sun J, Xia S, et al. Effects of different *Lactobacillus reuteri* on inflammatory and fat storage in high-fat diet-induced obesity mice model. J Funct Foods 2015;14:424-34. doi:10.1016/j.jff.2015.02.013.
27. Maulana H, Ridwan A. High-fat diets-induced metabolic disorders to study molecular mechanism of hyperlipidemia in rats. 3BIO 2021;3(2):92-105. doi:10.5614/3bio.2021.3.2.5.
28. Fleishman JS, Kumar S. Bile acid metabolism and signaling in health and disease: Molecular mechanisms and therapeutic targets. Signal Transduct Target Ther 2024;9(1):97. doi:10.1038/s41392-024-01811-6.
29. Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. Mol Metab 2021;50:101238. doi:10.1016/j.molmet.2021.101238.
30. McGill MR. The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI J 2016;15:817-28. doi:10.17179/excli2016-800.
31. Duan Y, Pan X, Luo J, et al. Association of inflammatory cytokines with non-alcoholic fatty liver disease. Front Immunol 2022;13. doi:10.3389/fimmu.2022.880298.

32. Huang Y, Chen H, Liu Q, et al. Obesity difference on association blood malondialdehyde level and diastolic hypertension in the elderly population: A cross-sectional analysis. Eur J Med Res 2023;28(1):44. doi:10.1186/s40001-022-00983-7.
33. Averina OV, Poluektova EU, Marsova MV, et al. Biomarkers and utility of the antioxidant potential of probiotic lactobacilli and bifidobacteria as representatives of the human gut microbiota. Biomedicines 2021;9(10):1340. doi:10.3390/biomedicines9101340.
34. Alkushi AG, Elazab ST, Abdelfattah-Hassan A, et al. Multi-strain-probiotic-loaded nanoparticles reduced colon inflammation and orchestrated the expressions of tight junction, NLRP3 inflammasome and caspase-1 genes in DSS-induced colitis model. Pharmaceutics 2022;14(6). doi:10.3390/pharmaceutics14061183.
35. El-Zayat SR, Sibaii H, Mannaa FA. Toll-like receptors activation, signaling, and targeting: An overview. Bull Natl Res Cent 2019;43(1):187. doi:10.1186/s42269-019-0227-2.
36. Zhao L, Shen Y, Wang Y, et al. *Lactobacillus plantarum* S9 alleviates lipid profile, insulin resistance, and inflammation in high-fat diet-induced metabolic syndrome rats. Sci Rep 2022;12(1):15490. doi:10.1038/s41598-022-19839-5.
37. Lei L, Zhao N, Zhang L, et al. Gut microbiota is a potential goalkeeper of dyslipidemia. Front Endocrinol (Lausanne) 2022;13:950826. doi:10.3389/fendo.2022.950826.
38. Cuevas-Sierra A, Ramos-Lopez O, Riezu-Boj JI, et al. Diet, gut microbiota, and obesity: Links with host genetics and epigenetics and potential applications. Adv Nutr 2019;10(suppl_1):S17-30. doi:10.1093/advances/nmy078.
39. Kameyama K, Itoh K. Intestinal colonization by a *Lachnospiraceae bacterium* contributes to the development of diabetes in obese mice. Microbes Environ 2014;29(4):427-30. doi:10.1264/jsme2.ME14054.
40. Villaseñor-Aranguren M, Rosés C, Riezu-Boj JI, et al. Association of the gut microbiota with the host's health through an analysis of biochemical markers, dietary estimation, and microbial composition. Nutrients 2022;14(23). doi:10.3390/nu14234966.